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THE EFFECT OF GAMMA RADIATION ON DDT RESISTANCE
IN DDT RESISTANT HOUSE FLIES, *MUSCA DOMESTICA* L.

by



Hugh Graham Philip

A THESIS

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FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "The Effect of Gamma Radiation on DDT Resistance in DDT Resistant House Flies, *Musca domestica* L.," submitted by Hugh Graham Philip in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Levels of the enzyme DDT-dehydrochlorinase (DDT-ase), which catalyzes the conversion of DDT to DDE in house flies, were traced during DDT resistant house fly development. Activity peaks were found in larvae just prior to pupation and in teneral adults. Very low activity was detected during the pupal stage and none in eggs and day-old larvae. Teneral adults were injected with the protein synthesis inhibitors actinomycin D, puromycin, or chloramphenicol. Alpha-glycerophosphate dehydrogenase (α -GPDH) synthesis, used as an indicator of protein synthesis inhibition, was reduced on the average 31% and 31% in males and only 11% and 10% in females analyzed 2 and 4 days after treatment. DDT-ase synthesis was reduced 30% in males after 2 days and 31% and 21% in females analyzed 2 and 4 days after treatment.

In 4-5 day-old larvae exposed as larvae less than 24 hours old to 6, 12, 18, or 24 Krads of gamma radiation, DDT-ase specific activity decreased as the radiation dose increased. After one additional day of development, DDT-ase specific activity decreased in both irradiated and non-irradiated larvae but the decrease was less in irradiated larvae exposed to greater doses of radiation. The significance of these results is discussed. DDT-ase activity in adults irradiated as 5-6 day-old pupae was unaffected by radiation. However gamma-irradiated adults showed increased tolerance to topically-applied DDT. Results indicate increased resistance of irradiated adults to DDT is not caused by increased levels of DDT-ase and possible alternative mechanisms for increased resistance are presented.

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AUTOBIOGRAPHICAL SKETCH

My introduction to entomology did not occur until 20 years after my birth in Kamloops, B. C. on September 18, 1945. After completing high school in Calgary in 1963, I enrolled at the University of Calgary in a pre-veterinary program because of my interest in agriculture and my agricultural background. However, a summer job with the Federal Department of Forestry and Rural Development turned my interest to insects and as a result I enrolled in the Faculty of Agriculture at the University of Alberta specializing in entomology. After receiving my B. Sc. (Agric.) in 1968, I immediately entered the Faculty of Graduate Studies in order to learn more about the insects and their world.

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I. INTRODUCTION

The vast amount of research being done on the mechanisms of insecticide resistance in insects was stimulated by the discovery in 1947 of a strain of house fly (*Musca domestica* L.) resistant to DDT (2,2-bis-(p-chlorophenyl)-1,1,1-trichloroethane) soon after the initiation of insect control programs using DDT (Wiesmann, 1947). The first significant discovery was independently made in 1950 by Sternburg and co-workers and by Perry and Hoskins showing that DDT resistant house flies detoxified DDT to DDE (2,2-bis-(p-chlorophenyl)-1,1-dichloroethane). Sternburg *et al.* (1953) showed that the metabolic process was enzymatic. The enzyme was later isolated and characterized as DDT-dehydrochlorinase (DDT-ase) (Sternburg *et al.* 1954, Lipke and Kearns 1959a, b). Lovell and Kearns (1959) showed a quantitative relationship between the presence of the enzyme and the degree of resistance to DDT in DDT resistant house flies. Kerr and co-workers (1957) were able to find low DDT-ase activity in a DDT susceptible strain of house flies. DDT-ase has not only been found in house flies but also in the Mexican bean beetle *Epilachna varivestis* (Tombes and Forgash, 1961), the corn earworm *Heliothis zea* and Polyphemus moth *Antheraea polyphemus* (Khan, 1969), and in the mosquitoes *Aedes aegypti* (Kimura and Brown, 1964), *Culex fatigans* and *C. tarsalis* (Kimura *et al.*, 1965). O'Brien (1967) reported that house fly DDT-ase can metabolize deuterio-DDT and p,p'-DDT but not o-chloro-DDT, whereas *Aedes aegypti* DDT-ase can metabolize o-chloro-DDT and p,p'-DDT but not deuterio-DDT.

Dehydrochlorination of DDT to DDE is only one of six genetically controlled mechanisms of house fly resistance to DDT. The genes responsible for these resistance mechanisms are on chromosomes II, III, and V, following the linkage groups of Wagoner (1967). DDT-ase is produced by a single semi-dominant gene *Deh* on chromosome II (Lovell and Kearns, 1959; Hoyer and Plapp, 1966; Sawicki and Farnham, 1967). Oppenoorth (1965a, b) found DDT-ases which differed in activity and substrate specificity produced by alleles of the *Deh* gene. He investigated the resistance mechanisms of three strains of house flies and found one strain to have a highly active DDT-ase whereas the other two strains had DDT-ases of low activity which contributed little to DDT resistance. One strain having a DDT-ase of low activity had a resistance factor on chromosome V which could be overcome by the synergist sesamex (2(3,4-methylenedioxyphenoxy)-3,6,9-trioxaundecane) but was not inhibited by the common DDT-ase inhibitors DMC (bis-(p-chlorophenyl)-methylcarbinol), F-DMC (bis-(p-chlorophenyl)-trifluoromethylcarbinol), and WARF-anti-resistant (Wisconsin Alumni Research Foundation). This gene, labelled *DDT-md*, was later found to control the microsomal detoxification of DDT (Oppenoorth and Houx, 1968; Gil *et al.*, 1968) and did not involve dehydrochlorination. Chromosome III has four genes which contribute to DDT resistance in house flies. Gene *kdr* is responsible for resistance to knockdown but the mechanism is unknown (Milani, 1955). Grigolo and Oppenoorth (1966) found that alleles of gene *Deh* producing moderately active DDT-ases enhanced the effect of gene *kdr*. An incompletely recessive gene labelled *r-DDT* found in a Japanese strain of house fly is suspected of being responsible for the low nerve sensitivity to

DDT exhibited by this strain (Tsukamoto *et al.*, 1965). Milani (1960) and Hoyer and Plapp (1966) found resistance to DDT in the Orlando house fly strain to be controlled by a recessive gene *kdr-0*, the mechanism of which is unknown but does not involve dehydrochlorination. The *kdr-0* gene confers resistance to a wide range of insecticides in house flies whereas the *Deh* gene confers resistance to only those compounds which are sterically similar to DDT. Sawicki and Farnham (1969) found a gene they labelled *Pen* which delayed knockdown by slowing down the entry of DDT into flies of the diazinon-selected SKA house fly strain.

Purification of house fly DDT-ase was first attempted by Sternburg *et al.* (1954) and Moorefield (1956). A more refined method was developed later by Lipke and Kearns (1959a, b, 1960). DDT-ase has an absolute requirement for reduced glutathione and no other -SH containing compounds have been found which activate this enzyme. Dinamarca and co-workers (1969), using purified house fly DDT-ase, found that the native enzyme was formed by four monomers each with a molecular weight of 30,000. Dimers and monomers were inactive; trimers and tetramers were active. The optimum molecular weight was 120,000 as larger aggregates were less active than the tetramer. DDT induced aggregation of the monomers. Glutathione was required for enzyme activity as it prevented the dissociation of the enzyme into monomers and polypeptides of lower molecular weights. The mechanism involved was not established but the authors speculated that DDT may form a charge-transfer complex with DDT-ase in much the same way as it does with nerve axon protein (O'Brien and Matsumura, 1964). Based

on the amount of soluble protein in various tissues of DDT resistant house flies, high levels of DDT-ase were found in the brain and fat body, intermediate levels in the cuticle, muscle, and haemolymph, with little or none in the ovary and intestinal tract (Miyake, Kearns, and Lipke, 1957). Moorefield and Kearns (1957) studied the levels of DDT-ase during DDT resistant house fly development and based DDT-ase activity on the amount of DDE produced by homogenates of 200 individuals. They found the enzyme quantitatively increased during the larval stage, decreased markedly at pupation, and remained constant throughout the pupal and adult stages.

II. STATEMENT OF THE PROBLEM

This present study attempts to show the effects, if any, of gamma radiation on DDT resistance in DDT resistant house flies. The mode of action of gamma radiation on DDT resistance is also investigated using one of the six genetically controlled DDT resistance mechanisms found in different strains of house flies. The mechanism studied was the dehydrochlorination of DDT to DDE which is catalyzed by DDT-dehydrochlorinase. Restricting study to only a single enzyme system would lend itself to a more straightforward experimental approach.

If Moorefield and Kearns (1957) had expressed DDT-ase activity as the amount of DDE produced per mg soluble protein, would the pattern of DDT-ase production they traced during house fly development remain the same? To answer this question, their experiment was repeated in this current study expressing DDT-ase activity on a per mg soluble protein basis. The results obtained from this experiment would aid in selecting those stages of house fly development to irradiate in order to show if gamma radiation had any effect on the levels of DDT-ase and on DDT resistance in house flies.

Therefore the purpose of this study is:

1. To study the changes in DDT-ase activity during DDT resistant house fly development;
2. To study the influence of various doses of gamma radiation on DDT-ase activity; and
3. To study the effect of gamma radiation on the resistance of DDT resistant house flies to DDT.

III. EXPERIMENTAL METHODS

1. Rearing of *Musca domestica* L.:

The DDT resistant house fly strain (#79 DR-125 + 6) used in this study was obtained from the Experimental Farm, Canada Department of Agriculture, Ottawa. Adults were kept in cotton mesh-covered 20 cm x 20 cm x 30 cm wooden framed cages at 24 ± 1 C and 55-60% relative humidity. Adults were fed *ad libitum* on water and on granulated sugar. A petri dish containing cotton soaked in canned milk was provided for oviposition. Eggs were transferred to quart sealers 2/3 filled with bran mixed with canned milk (1 : 2 w/w) on which the larvae were reared. Pupae were collected and placed in rearing cages prior to adult emergence. Samples of eggs, larvae, and pupae were thoroughly washed with distilled water and dried before being frozen and stored at -10 C for use at a later date.

2. Determination of soluble protein:

For determining the amount of soluble protein per insect at each stage of development, 100 eggs, 100 day-old larvae, and 20 1-2 day-old larvae were assayed per sample. For older larvae, pre-pupae, pupae, and adults, 4 insects were individually assayed for each age level sampled. Two males and 2 females were assayed for each sample of adults. The method of Lowry *et al.* (1951) was used to determine the amount of soluble protein. This method also measures free amino acid content and tryptophan, tyrosine, guanine, and phenolic compounds give color reactions with the Folin phenol reagent. Sucrose has also been shown

to interfere with color development (Gerhardt and Beevers, 1968).

All insects were homogenized in 10^{-4} M phosphate buffer pH 7.4 using a 2 ml Pyrex hand homogenizer. After centrifugation of the homogenate at $12,350 \times g$ at 2 C for 30 minutes in a Sorvall RC2-B refrigerated centrifuge, the precipitate was discarded and the supernatant used for soluble protein determination. Crystalline bovine serum albumin was used as the standard. Color development of the reaction mixture was measured at 500 nm using a Beckman DU-2 spectrophotometer.

3. Determination of DDT-dehydrochlorinase activity:

The different age levels of house fly development were sampled the same as for soluble protein determinations. DDT-ase activity was determined using gas-liquid chromatography after the method of Oppenoorth and Voerman (1965). Acetone powders of insect material were incubated in a nitrogen atmosphere at 37 ± 1 C for one hour with 2 μ moles of 99.9% p,p'-DDT (City Chemical Corporation, N. Y.) suspended in 50 μ l of dimethylsulfoxide in the presence of a buffer solution containing phosphate buffer pH 7.4, reduced glutathione (Sigma Chemical Company), and neutralized EDTA-sodium. The reaction was stopped by adding the mixture to a saturated sodium sulfate solution. The remaining DDT and any DDE produced were extracted with 10 mls of a 2:1 cyclohexane-propanol solution. Two μ moles of DDT were sufficient to saturate any DDT-ase present and maximum conversion of DDT to DDE was less than 5%.

To determine the amount of DDE produced, one microliter of the extract was injected into a Varian Aerograph Series 1200 gas-liquid chromatograph with an electron-capture tritium detector and equipped

with a Sargent recorder model SR and later with a Disc Model 224-4 recorder (Disc Instruments, Inc., Santa Ana, Calif.). The 3 mm x 60 cm Pyrex column was packed with 6% QF-1 and 4% SE-30 on Chromosorb W 60/80 mesh. Nitrogen was the carrier gas at a flow rate of 22 mls per minute. The temperatures of the column, injector, and detector were 188 C, 220 C, and 210 C respectively. One microliter of one part per million DDE (Geigy A. G. Basal) and one part per million DDT standard solution was injected every 8-12 sample injections to check for any decrease in detector sensitivity due to contamination of the tritium foil. For each set of DDT-ase assays a control reaction mixture containing DDT, reduced glutathione, neutralized EDTA, and buffer was incubated without house fly material to check for any DDE present in the reactants, the cyclohexane-propanol, and to check for any non-enzymatic conversion of DDT to DDE during incubation. The heights of the DDE standard peak and of the DDE from samples were kept within 1/3 of each other (Fig. 1). The areas of the peaks were estimated by multiplying the heights by the width at $\frac{1}{2}$ the height of the peak.

4. Antibiotic studies:

4.1 Application of antibiotics:

To determine whether or not DDT-ase is synthesized in adults, duplicate batches of 50 newly emerged flies were treated with the protein synthesis inhibitors puromycin, actinomycin D, or chloramphenicol. The antibiotics were dissolved in 0.9% saline pH 7.0. Flies were lightly anesthetized with CO₂ and kept cool during treatment. Each fly was treated with 1 µg of antibiotic because 1 µg was sufficient

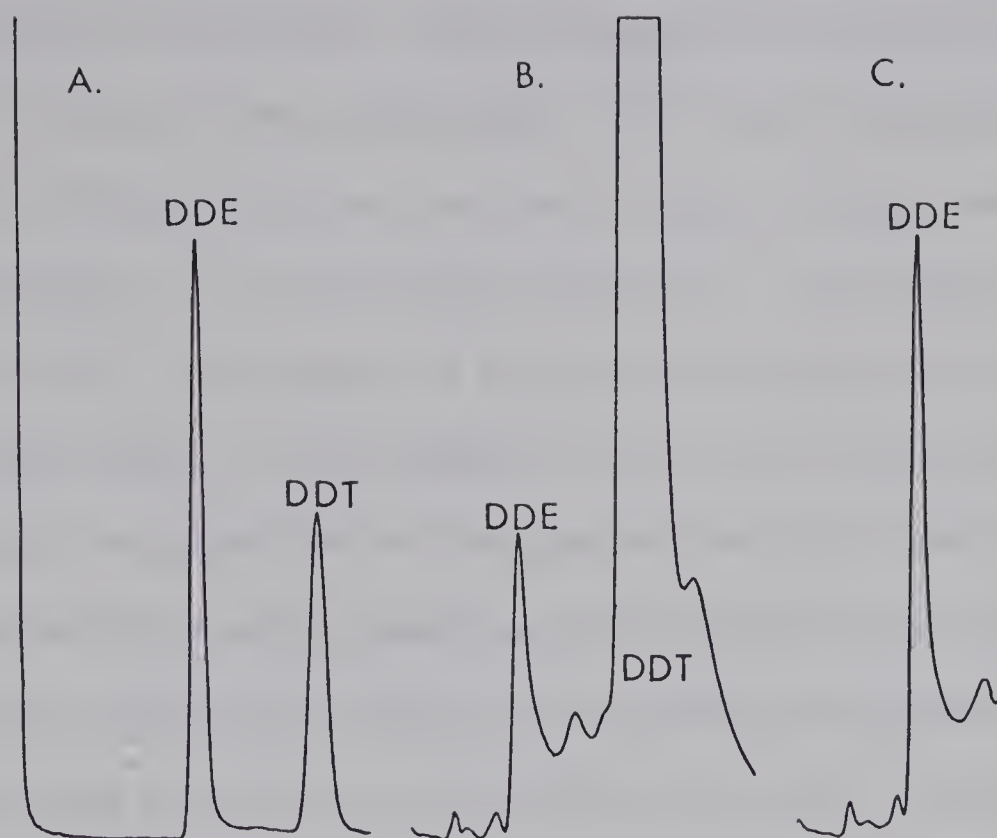


Fig. 1. Examples of GLC estimations of DDT-metabolism in DDT resistant house flies, *Musca domestica* L.

- A. Standard peaks of 1 ppm DDE and 1 ppm DDT.
- B. Reactants (DDT, reduced glutathione, EDTA, and phosphate buffer) incubated without house fly homogenate.
- C. Typical DDE peak after incubation of reactants with house fly homogenate.

to produce a significant effect on α -GPDH specific activity and not cause immediate death of the flies. One microgram of antibiotic was injected into the thorax of each fly using a ISCO Model M microapplicator with a $\frac{1}{4}$ cc tuberculin glass syringe and an 18 gauge syringe needle calibrated to dispense 1 μ l of antibiotic solution. The needle was shortened to 1 cm and a 3 cm length of 2 mm plastic tubing was securely fitted on the distal end. A 2 cm length of 2 mm glass tubing drawn to a very fine point was inserted in the open end of the plastic tubing. The treated flies were placed in gauze-covered quart sealers supplied with cotton soaked in a 20% (w/v) sucrose-water solution and kept in a rearing room at 24 ± 1 C and 55-60% R.H. with a 14-10 light-dark photoperiod. Samples of treated and untreated flies were taken 2 and 4 days after treatment.

4.2 Assay of α -glycerophosphate dehydrogenase:

Alpha-glycerophosphate dehydrogenase (α -GPDH) activity was determined after the method of Marquardt and Brosemer (1966). From each sample of treated flies, 2 males and 2 females were separately homogenized in a buffer solution of 20 mM EDTA, 0.3 M sucrose, and 0.1 M Tris pH 7.8 and centrifuged in the cold at $12,350 \times g$ for 40 minutes. The precipitate was discarded and 0.1 ml of the supernatant was added to 1.0 ml of the reaction mixture consisting of 33 mM α -glycerophosphate, 2 mM NAD^+ , 50 mM Tris buffer pH 7.8, and 5 mM EDTA. The rate of change in NADH concentration was measured at 340 nm using a Beckman DU-2 spectrophotometer equipped with a dual thermospacer which maintained the reaction temperature at 30 ± 1 C. α -GPDH activity

was expressed as the μ moles of NADH generated per fly in the first minute of the reaction. Endogenous production of NADH was checked by running controls which contained α -glycerophosphate, buffer, and homogenate or NAD^+ , buffer, and homogenate.

5. Irradiation studies:

5.1 Irradiation of larvae and pupae:

Irradiations were carried out using a Gammacell Cobalt-60 chamber (Atomic Energy of Canada) under constant conditions at a dose rate of 6 Krads of gamma radiation per minute. Pupae 5-6 days old were irradiated to show any effects gamma radiation may have on the production of DDT-ase in tenerals and older adults. One hundred day-old larvae were placed in petri dishes lined with moistened filter paper and irradiated with 6, 12, 18, and 24 Krads of gamma radiation. About 1000 5-6 day-old pupae were placed in small glass jars and irradiated with the above doses of gamma radiation. Irradiated larvae were transferred to rearing jars immediately after exposure; irradiated pupae were immediately placed in rearing cages. Control batches of larvae and pupae were also handled as above except they were not placed in the irradiation chamber. The amount of soluble protein and DDT-ase was determined for each age level of larvae and adults sampled.

5.2 Determination of LD_{50} :

Six to seven day-old irradiated and non-irradiated adults were anesthetized with CO_2 and treated with 100% p,p'-DDT dissolved in acetone at concentrations ranging from 0.001% to 2.5% (w/v). An ISCO Model M

microapplicator equipped with a $\frac{1}{4}$ cc tuberculin syringe was calibrated to dispense 1 μ l of acetone DDT solution to the dorsal thorax of each fly. Forty unsexed flies were treated per concentration and incubated in quart jars supplied with a 20% (w/v) sucrose-water solution in an incubator set at 26.7 C and 60% R. H. Mortality counts were taken after 24 hours. Flies were counted as dead if they were immobile or had difficulty in maintaining balance. LD50 values and linear regression equations were calculated using a modified computer program adapted from a probit analysis program (Daum and Killcreas, 1966) and a correlation and plotting program (Yanda, 1968).

IV. RESULTS

1. Levels of DDT-dehydrochlorinase during house fly development:

Table 1 shows the changes in DDT-ase activity for each age level sampled during DDT resistant house fly development. DDT-ase activity is expressed as the nanomoles DDE produced per hour per insect, per milligram soluble protein, and per milligram insect weight. No DDT-ase activity was detected in eggs or in larvae less than one day old. The amount of active DDT-ase increases during the remainder of larval development, reaching a peak just prior to pupation. During pupation the level of DDT-ase drops sharply to a low level which is maintained throughout the pupal stage. Upon emergence of the adults the amount of DDT-ase per insect increases sharply and continues to increase with aging. The specific activity (amount of DDE produced per mg soluble protein) of DDT-ase reaches a peak in 3-4 day-old larvae then decreases with the onset of pupation, following the same pattern throughout the remainder of development as described for the amount of active DDT-ase per insect. The level of DDT-ase based on the weight of the individuals when analyzed also displays the same pattern as above with peaks of activity in late larval and early adult life, and low activity during the pupal stage.

The next problem investigated was whether DDT-ase synthesized in the larval stage was inhibited during the pupal stage and reactivated upon adult eclosion or whether larval DDT-ase was broken down during pupation and resynthesized in young adults. The antibiotics puromycin, actinomycin D, and chloramphenicol are common protein synthesis

Table 1. Changes in DDT-dehydrochlorinase activity during DDT resistant house fly development*

Stage & age (days)	Soluble protein (mg/insect)	Nanomoles DDE produced/hour		
		/insect $\pm$ S.D.	/mg sol. prot. $\pm$ S.D.	/mg insect weight $\pm$ S.D.
Egg		-	-	-
Larva				
0-1		-	-	-
1-2	0.020	0.25 $\pm$ 0.00	12.50 $\pm$ 0.00	0.720 $\pm$ 0.00
2-3	0.442	6.87 $\pm$ 1.19	15.54 $\pm$ 2.69	0.840 $\pm$ 0.145
3-4	0.370	10.14 $\pm$ 0.39	27.41 $\pm$ 1.05	0.686 $\pm$ 0.026
4-5	1.326	17.50 $\pm$ 4.19	13.20 $\pm$ 3.70	0.894 $\pm$ 0.220
Pre-pupa	1.546	13.67 $\pm$ 6.12	8.84 $\pm$ 4.62	0.759 $\pm$ 0.264
Pupa				
0-1	1.674	7.91 $\pm$ 5.65	4.73 $\pm$ 3.38	0.464 $\pm$ 0.332
2-3	1.568	1.20 $\pm$ 0.67	0.77 $\pm$ 0.43	0.071 $\pm$ 0.040
4-5	1.485	6.01 $\pm$ 0.48	4.05 $\pm$ 0.32	0.332 $\pm$ 0.029
Teneral	1.366	13.95 $\pm$ 7.28	10.21 $\pm$ 5.33	0.930 $\pm$ 0.485
Adult				
1-3	0.928	13.53 $\pm$ 1.40	14.58 $\pm$ 1.51	1.178 $\pm$ 0.116
3-4	0.880	15.32 $\pm$ 7.01	17.41 $\pm$ 7.97	1.253 $\pm$ 0.540
4-5	0.719	17.45 $\pm$ 4.41	24.27 $\pm$ 6.13	1.745 $\pm$ 0.340

* At least 2 replicates were made of each age level sampled.

All samples were frozen prior to analysis.

inhibitors. If young adults synthesize DDT-ase treating tenerals with one of these antibiotics should lower adult DDT-ase levels. The specific activity of α -glycerophosphate dehydrogenase (α -GPDH) was determined to test whether protein synthesis was being inhibited in treated flies since it has been shown that the amount of α -GPDH increases sharply upon adult emergence coinciding with development of adult flight muscle (Rechsteiner, 1970). Treated adults analyzed 2 and 4 days after treatment showed some inhibition of α -GPDH synthesis (Tables 2A, B). Males showed the greatest percent decrease in α -GPDH activity for each antibiotic. Wide variation was found in DDT-ase activity per treated and untreated adults (Table 3A, B). Two days after treatment the average percent loss of DDT-ase specific activity in males was 30.3% whereas α -GPDH specific activity decreased on the average 31.3%. The corresponding values for treated females assayed after 2 days are 31.0% and 10.7%. After 4 days DDT-ase specific activity decreased 20.6% in females whereas α -GPDH specific activity decreased 10.1%. The specific activity of male α -GPDH had decreased 30.6% 4 days after treatment. No males were available in the 4-day samples for DDT-ase assays as those collected were previously used for determining soluble protein content and α -GPDH activity. Samples of treated flies were collected only up to 4 days after treatment since increasing numbers of adults were unable to fly, becoming much less active and eventually dying on day 5 or 6. Only actively flying adults were collected. Untreated flies, injected with 1 μ l of saline, lived at least 2 weeks with little mortality. Two days after treatment, the apparent soluble protein content of treated flies was greater than that of untreated

Table 2. Effect of antibiotics on α -glycerophosphate dehydrogenase activity in adult house flies*

A. Changes in the amount of active α -GPDH.

Antibiotic	Sex	μ Moles NADH generated/minute/fly			
		2 days	% loss	4 days	% loss
Control	Male	0.230		0.281	
	Female	0.240		0.233	
Puromycin	Male	0.190	17.5	0.217	22.7
	Female	0.217	9.5	0.220	5.7
Actinomycin D	Male	0.224	2.3	0.209	25.4
	Female	0.252	0.0	0.211	9.3
Chloramphenicol	Male	0.212	7.5	0.236	15.8
	Female	0.226	6.0	0.253	0.0

* Averaged results of two experiments run simultaneously.

Teneralis injected with 1 μ g of antibiotic and analyzed 2 and 4 days later.

B. Changes in the specific activity of α -GPDH.

Antibiotic	Sex	μ Moles NADH generated/min./mg soluble protein			
		2 days	% loss	4 days	% loss
Control	Male	0.338		0.480	
	Female	0.209		0.232	
Puromycin	Male	0.213	37.0	0.358	25.4
	Female	0.193	7.7	0.217	6.5
Actinomycin D	Male	0.260	23.1	0.287	40.2
	Female	0.200	4.3	0.196	15.5
Chloramphenicol	Male	0.224	33.7	0.354	26.3
	Female,	0.167	20.1	0.213	8.2

Table 3. Effect of antibiotics on DDT-dehydrochlorinase activity
in adult house flies*

A. Changes in the amount of active DDT-ase.

Antibiotic	Sex	Nanomoles DDE produced/hour/fly**			
		2 days	% loss	4 days	% loss
Control	Male	15.41 $\pm$ 5.61			
	Female	28.77 $\pm$ 7.89		17.96 $\pm$ 2.59	
Puromycin	Male	12.91 $\pm$ 3.59	16.2		
	Female	22.18 $\pm$ 6.91	22.9	13.87 $\pm$ 5.74	22.8
Actinomycin D	Male	14.32 $\pm$ 2.17	7.1		
	Female	18.32 $\pm$ 4.35	36.3	17.52 $\pm$ 4.58	2.5
Chloramphenicol	Male	15.47 $\pm$ 1.58	0.0		
	Female	25.58 $\pm$ 6.63	11.1	15.04 $\pm$ 3.89	16.3

* Averaged results of two experiments run simultaneously.

Teneralis injected with 1 μ g of antibiotic and analyzed 2 and 4 days later.

** Mean $\pm$ standard deviation.

B. Changes in the specific activity of DDT-ase.

Nanomoles DDE produced/hour/mg soluble protein**					
Antibiotic	Sex	2 days	% loss	4 days	% loss
Control	Male	22.66 ± 8.25			
	Female	25.01 ± 6.86		17.87 ± 2.58	
Puromycin	Male	14.51 ± 4.02	36.0		
	Female	19.77 ± 6.40	26.5	13.65 ± 5.65	23.6
Actinomycin D	Male	16.55 ± 2.44	27.0		
	Female	14.52 ± 4.10	42.0	16.28 ± 4.25	8.9
Chloramphenicol	Male	16.35 ± 1.64	27.9		
	Female	18.91 ± 4.67	24.4	12.66 ± 1.49	29.2

** Mean ± standard deviation.

flies (Table 4). Two days later the apparent soluble protein content of treated flies had almost returned to normal.

2. Effect of gamma radiation on DDT-ase levels in larvae:

No statistically significant difference at the 5% level was found in the amount or in the specific activity of DDT-ase between larvae 4-5 and 5-6 days old irradiated at less than 24 hours old and non-irradiated larvae of the same age (Table 5). In irradiated 4-5 day-old larvae the amount of active DDT-ase is less than in control larvae except in 6 Krad-irradiated larvae which have more. After an additional day of development the amount of active enzyme decreased 50% in control and 6 Krad-irradiated larvae with no change in 12 Krad irradiated larvae and almost a 50% increase in larvae exposed to 18 Krads of gamma radiation. DDT-ase specific activity decreased as radiation dose increased in larvae 4-5 days old. One day later the specific activity was greater in irradiated larvae than in control larvae. In other words, specific activity decreased less in irradiated larvae. Larvae exposed to 24 Krads of radiation died before pupation whereas larvae exposed to smaller doses managed to pupate but no adults emerged. There was little difference in the soluble protein content of non-irradiated 4-5 day-old larvae and 4-5 day-old larvae irradiated at less than 24 hours old. The same results were also observed between non-irradiated 5-6 day-old larvae and 5-6 day-old larvae irradiated at less than 24 hours old (Table 6).

Table 4. Effect of antibiotics on the apparent soluble protein[†]
content of adult house flies*

Antibiotic	Sex	Mg soluble protein/fly $\pm$ S.D.	
		2 days	4 days
Control	Male	0.680 $\pm$ 0.060	0.585 $\pm$ 0.077
	Female	1.150 $\pm$ 0.070	1.005 $\pm$ 0.015
Puromycin	Male	0.890 $\pm$ 0.077	0.606 $\pm$ 0.003
	Female	1.122 $\pm$ 0.104	1.016 $\pm$ 0.197
Actinomycin D	Male	0.865 $\pm$ 0.077	0.728 $\pm$ 0.187
	Female	1.262 $\pm$ 0.268	1.077 $\pm$ 0.104
Chloramphenicol	Male	0.946 $\pm$ 0.031	0.666 $\pm$ 0.118
	Female	1.353 $\pm$ 0.238	1.188 $\pm$ 0.293

* Results of two experiments run simultaneously. Adults analyzed
2 and 4 days after treatment.

[†] The amount of soluble protein reported here includes both soluble
protein and some free amino acids.

Table 5. Effect of gamma radiation on DDT-dehydrochlorinase activity in 4-5 and 5-6 day-old larvae*

A. Changes in the amount of active DDT-ase.

Dose (Krads)	Nanomoles DDE produced/hour/larva			
	4-5 day-old		5-6 day-old	
	Mean	Range	Mean	Range
0	17.90	4.44 to 31.66	8.87	2.48 to 15.62
6	25.83	20.30 to 31.48	11.70	0.68 to 23.30
12	8.86	3.83 to 17.81	8.75	1.06 to 15.62
18	9.98	2.63 to 21.73	13.20	4.70 to 22.30
24	3.83	1.67 to 7.91		

B. Changes in the specific activity of DDT-ase.

Dose (Krads)	Nanomoles DDE produced/hour/mg soluble protein			
	4-5 day-old		5-6 day-old	
	Mean	Range	Mean	Range
0	17.29	4.29 to 30.11	3.38	0.94 to 5.96
6	15.96	12.55 to 19.46	4.83	0.28 to 9.63
12	10.80	4.67 to 21.72	5.68	0.69 to 10.14
18	7.77	2.05 to 16.91	7.10	2.53 to 11.90
24	4.63	2.02 to 9.56		

* Results from two experiments. Larvae irradiated less than 24 hours after hatching.

Table 6. Effect of gamma radiation on the soluble protein content of 4-5 and 5-6 day-old larvae*

Dose (Krad/s)	Mg soluble protein larva $\pm$ S.D.	
	4-5 day-old	5-6 day-old
0	1.04 $\pm$ 0.17	2.62 $\pm$ 0.48
6	1.62 $\pm$ 0.53	2.42 $\pm$ 0.10
12	0.82 $\pm$ 0.38	1.54 $\pm$ 0.34
18	1.29 $\pm$ 0.17	1.86 $\pm$ 0.55
24	0.83 $\pm$ 0.28	

* Results of two experiments run simultaneously. Larvae irradiated less than 24 hours after hatching.

3. Effect of gamma radiation on DDT-ase activity in adults:

Gamma radiation had no significant effect at the 5% level on the levels of DDT-ase in adults irradiated as 5-6 day-old pupae (Table 7). The mean amount and mean specific activity of the enzyme per adult increased with aging of both control and irradiated adults. Control and irradiated adults analyzed at the same age showed no significant difference in DDT-ase levels. Adults which emerged from irradiated 5-6 day-old pupae tended to have more soluble protein than non-irradiated adults (Table 8). Soluble protein content of irradiated adults generally increased as the radiation dose increased.

4. Effect of gamma radiation on adult resistance to DDT:

Table 9 gives the LD₅₀'s (μg DDT/mg insect weight) and linear regression equations of 6-7 day-old adults exposed as 5-6 day-old pupae. The linear regression equations give the slopes of the probit vs. log dose curves for each LD₅₀ test and provide a means for calculating the LD₃₀, LD₇₀ and LD₉₀ values. Tests were conducted on two separate generations of house flies. Test I results show that irradiation increased adult resistance to DDT, the greatest increase being in flies exposed to 12 and 18 Krads. In Test II radiation doses of 12 and 18 Krads again caused the greatest increase in DDT resistance but the increases were less than in Test I. Flies exposed to 6 Krads showed increased tolerance to DDT in Test I but slightly decreased tolerance in Test II. Flies exposed to 24 Krads also showed increased tolerance to DDT in Test I but showed no change in Test II. All doses of radiation completely stopped oviposition as no eggs were found in

Table 7. Effect of gamma radiation on DDT-dehydrochlorinase activity
in DDT resistant house flies*

A. Changes in the amount of active DDT-ase.

Nanomoles DDE produced/hour/fly $\pm$ S.D.

Dose (Krads)	Age (days)			
	0-1	1-2	2-3	3-4
0	8.08 $\pm$ 0.98	12.32 $\pm$ 1.88	14.27 $\pm$ 4.40	15.97 $\pm$ 4.75
6	12.46 $\pm$ 1.52	15.01 $\pm$ 2.78	13.83 $\pm$ 3.19	
12	12.09 $\pm$ 1.25			20.07 $\pm$ 6.38
18	9.52 $\pm$ 3.25		12.64 $\pm$ 1.43	15.09 $\pm$ 3.02
24	15.58 $\pm$ 6.85	14.42 $\pm$ 5.83	18.47 $\pm$ 4.92	20.42 $\pm$ 5.68

B. Changes in the specific activity of DDT-ase.

Nanomoles DDE produced/hour/mg soluble protein $\pm$ S.D.

Dose (Krads)	Age (days)			
	0-1	1-2	2-3	3-4
0	12.14 $\pm$ 1.35	16.83 $\pm$ 2.57	27.82 $\pm$ 8.85	26.44 $\pm$ 7.86
6	15.69 $\pm$ 1.92	23.09 $\pm$ 4.28	22.67 $\pm$ 5.32	
12	13.69 $\pm$ 1.42			27.20 $\pm$ 8.64
18	9.76 $\pm$ 3.33		16.39 $\pm$ 1.85	27.09 $\pm$ 5.42
24	15.52 $\pm$ 6.82	13.56 $\pm$ 5.48	25.87 $\pm$ 6.89	27.78 $\pm$ 7.73

* Data from two generations of flies irradiated as 5-6 day-old pupae.

Table 8. Effect of gamma radiation on the soluble protein content of adult flies at various ages*

Dose (Krad)	Mg soluble protein/adult $\pm$ standard deviation			
	Age (days)			
	0-1	1-2	2-3	3-4
0	0.666 $\pm$ 0.184	0.732 $\pm$ 0.137	0.513 $\pm$ 0.012	0.604 $\pm$ 0.077
6	0.794 $\pm$ 0.114	0.650 $\pm$ 0.137	0.610 $\pm$ 0.054	
12	0.883 $\pm$ 0.134			0.738 $\pm$ 0.170
18	0.975 $\pm$ 0.054		0.771 $\pm$ 0.054	0.557 $\pm$ 0.144
24	1.004 $\pm$ 0.212	1.063 $\pm$ 0.141	0.714 $\pm$ 0.079	0.735 $\pm$ 0.109

* Results from two experiments on separate generations of flies irradiated as 5-6 day-old pupae.

Table 9. Effect of gamma radiation on DDT resistance in DDT resistant house flies*

Dose (Krad)	Test	LD ₅₀ ± 2 S.D. (µg DDT/mg insect weight)***	Linear regression equation**
0	I	0.179 ± 0.039	y = 6.224 + 1.864x
	II	0.115 ± 0.039	y = 6.278 + 1.377x
6	I	0.237 ± 0.093	y = 5.373 + 0.801x
	II	0.108 ± 0.064	y = 6.322 + 1.444x
12	I	0.426 ± 0.282	y = 5.365 + 1.465x
	II	0.191 ± 0.058	y = 6.008 + 1.150x
18	I	0.260 ± 0.222	y = 5.771 + 1.565x
	II	0.220 ± 0.063	y = 6.143 + 1.789x
24	I	0.204 ± 0.047	y = 6.080 + 2.026x
	II	0.115 ± 0.035	y = 6.495 + 1.662x

* Six to seven day-old adults irradiated as 5-6 day-old pupae.

Results of tests conducted on two non-successive generations.

** Probit = a + b log % DDT.

*** Insect weight estimated by weighing a pool of 40 flies.

cages of irradiated flies supplied with egg-laying media. No attempt was made to study the effects of sub-sterilizing doses of radiation on the levels of DDT-ase in irradiated adults and subsequent progeny.

V. DISCUSSION

The changes in levels of DDT-ase during house fly development found in this study do not fully coincide with those obtained by Moorefield and Kearns (1957). In addition to a peak of high DDT-ase activity in last instar larvae, my results show a second peak during early adult life which was not detected by Moorefield and Kearns (1957). Discovery of this second peak of DDT-ase activity may be the result of using a more sensitive technique for detection of DDE. Lipke and Kearns (1960) suggested that had DDT-ase activity been based on soluble protein content the differences obtained by Moorefield and Kearns would have been less significant. My results show otherwise. A similar pattern of development was also traced for alcohol dehydrogenase in *Drosophila melanogaster* (Ursprung *et al.*, 1968) and for DDT-ase in the Mexican bean beetle, *Epilachna varivestis* (Tombes and Forgash, 1961).

The sharp decrease in the level of DDT-ase at pupation is to be expected because of the extensive tissue re-organization occurring at this time. Simultaneously there is extensive synthesis of structural proteins for building adult tissues which replace the degenerating larval structures. DDT-ase may be broken down to provide amino acids thus reducing the amount of active enzyme. Moorefield and Kearns (1957) suggested that during pupation, major sites of DDT metabolism are broken down and detoxification systems are disrupted resulting in low levels of DDT-ase activity. Alternatively the low DDT-ase activity during the pupal stage may also be caused by some

factor or factors released during pupation which inhibit the enzyme. The most active form of DDT-ase is a tetramer with a molecular weight of 120,000 (Dinamarca *et al.*, 1969) and inhibition of any one of the monomers will reduce enzyme activity. The inhibitor(s) may also prevent the aggregation of the monomers in the presence of DDT to form the active tetramer, or may attack the active site of the enzyme thus completely inhibiting the enzyme. Upon emergence of the adults, inhibition may be destroyed and the level of active DDT-ase increases.

The results of experiments using the antibiotics puromycin, actinomycin D, and chloramphenicol tend to favor the suggestion that perhaps DDT-ase is broken down during pupation and resynthesized again in early adult life. α -GPDH was selected as an indicator of protein synthesis inhibition because just prior to adult eclosion the amount of active α -GPDH increases rapidly. This sharp increase coincides with the development of adult flight muscles since α -GPDH is very important in flight muscle metabolism. Brosemer (1965a) found a 20 fold increase in α -GPDH activity soon after the imaginal molt of the migratory grasshopper, *Schistocerca vaga*. Brosemer (1965b) found that after injecting puromycin and actinomycin D into the thoracic muscles of newly molted male differential grasshoppers (*Melanoplus differentialis*) α -GPDH activity was reduced 20% and 40% by puromycin and 44% by actinomycin D. My results also show comparable reductions in α -GPDH activity after injecting an antibiotic into the thoracic muscles of teneral house flies. The average percent decrease in DDT-ase activity in treated flies was as great or greater than the average percent decrease in α -GPDH activity. The reduction of DDT-ase

activity in adults treated with antibiotics indicates that some DDT-ase is resynthesized in young adults but further research is required to determine what happens to DDT-ase during the pupal stage. Treatment of teneralis with a labelled amino acid could provide conclusive evidence that indeed all adult DDT-ase is synthesized in teneralis. Had DDT-ase been inhibited during the pupal stage, no difference in the levels of active enzyme would have been observed between treated and untreated flies.

Flies treated with antibiotics showed increased levels of soluble protein 2 days after treatment (Table 4). Since the Lowry method of protein determination also measures free amino acids, this apparent increase in soluble protein content may be a measurement of the increased free amino acid pool due to the inhibition of protein synthesis. Certain amino acids such as tryptophan and tyrosine may cause greater color development when free than when bound in a protein, hence increasing the apparent soluble protein content. Four days after treatment, the apparent soluble protein content of treated flies had returned almost to normal indicating that the effects of the antibiotics had worn off allowing normal protein synthesis.

Casarett (1968) stated that enzyme activity is not very radio-sensitive. Alteration of enzyme activity occurs with radiation doses much higher than those required to produce mutations, or chromosome breakage, or to prevent cell growth and division. Low doses of radiation may delay DNA, RNA, and protein synthesis but high doses depress synthesis. This depression is caused by alteration of DNA such that it cannot act as an RNA template for m-RNA production, hence protein

is not synthesized. No work has been done with insects on the effects of ionizing radiation on enzymes *in vivo*.

Most studies on exposure of enzymes to ionizing radiation have involved irradiating preparations of purified enzymes and noting changes in their physical and chemical properties. A comprehensive review article on the effects of ionizing radiation on enzymes was written by Augenstine (1962), and the following discussion briefly outlines relevant information from that article. Enzymes are inactivated either by direct effects (ionization within or near the enzyme molecule itself) or by indirect effects (enzymes in solution reacting with products produced by the action of radiation on the solvent). The amount of inactivation depends on how the enzymes were prepared, i.e. whether dry, in pure solution, or in a heterogeneous system. Enzymes in dry preparations are much more radioresistant than enzymes in aqueous preparations. Since I investigated the effects of gamma radiation on DDT-ase *in vivo*, the following discussion considers only factors contributing to enzyme inactivation in heterogeneous systems. Augenstine states that radiation damage in cells is random as only 1 in 10^6 atoms are affected. The primary mechanism for enzyme inactivation is the reaction of the enzyme molecule with free radicals formed from ionized water. However a living cell contains many other molecules with which these radicals can react, thereby reducing the chance that one particular enzyme will react with the radicals. The free radicals can cause serious impairment of enzyme activity in several ways. Radiosensitive hydrogen and disulfide bonds can rupture altering the secondary and tertiary structure of an enzyme. Conformational changes

can prevent the attachment of the substrate to the active site of the enzyme. Also, alteration of side chains will have a detrimental effect on enzyme activity. Augenstine proposed that enzyme inactivation is the result of specific as opposed to indiscriminate modifications of the secondary structure of an enzyme molecule. He maintained that enzymes have areas of weak bonds such as hydrogen bonds which are crucial for the maintenance of the unique configuration of the enzyme. Rupture of all these bonds causes irreversible inactivation. However if some of these bonds are kept intact, enzyme activity can be restored. Therefore one can see the complexity of the events which can render an enzyme exposed to ionizing radiation partially or wholly inactive and the damage radiation can impart to the synthetic pathway of an enzyme molecule.

Gamma radiation had no statistically significant effect on the level of DDT-ase in 4-5 and 5-6 day-old larvae irradiated less than 24 hours after hatching. There was wide variation in the levels of DDT-ase among individual larvae exposed to the same radiation dose due to genetic variation and differences in the degree of radiation damage. However the mean amount and mean specific activity of DDT-ase per irradiated larva did show a response to radiation exposure. The lower level of active enzyme in 5-6 day-old larvae compared to 4-5 day-old larvae exposed as larvae less than 24 hours old to 6 Krads followed the pattern of DDT-ase development in non-irradiated larvae. The level of active enzyme decreases with the onset of pupation. The decrease in DDT-ase activity was less in irradiated larvae after an additional day of development, and this could be caused by reversible

damage to the enzyme itself or to the synthetic pathway of the enzyme molecule, producing a lag in enzyme production. As pupation approached, more enzyme molecules were being synthesized or more enzyme molecules were recovering from radiation damage. Any number of radiation-induced inactivation mechanisms could be responsible for the loss in total DDT-ase activity in irradiated larvae. Radiation may have induced structural modifications in the monomers which make up the active tetramer such that the secondary and tertiary structure of the tetramer were altered. The monomers may also have been so structurally modified that DDT-induced aggregation of the monomers to form the active enzyme molecule was impaired or retarded. The active center may have been damaged thus rendering the enzyme inactive. Radiation damage to other cell constituents could impair cellular metabolic activity and thereby indirectly retard DDT-ase production. Winstead and Reece (1970) suggested that gamma radiation damage to purified lactate dehydrogenase was caused by the breaking of bonds responsible for the tertiary structure. Ebhart and Heinicke (1967) suggested that high doses of gamma radiation prevented sucrose from attaching as strongly to the enzyme dextran sucrose.

Although larvae exposed to 6, 12, and 18 Krads of gamma radiation managed to pupate, no adults emerged. Exposure of larvae to 24 Krads not only resulted in a severe loss of active DDT-ase but also in premature death as all died before pupation. Henneberry (1963) reported that few *Drosophila melanogaster* larvae survived to the pupal stage after exposure to 8 and 16 Krads and that no adults emerged from those that pupated. Henneberry (1967) found also that almost 100% mortality

occurred in the pupal stage with no adult emergence when day old *D. melanogaster* larvae were irradiated with 4.0 and 6.2 Krads of gamma radiation.

Unlike in larvae, the amount and specific activity of DDT-ase were unaffected in adults irradiated as 5-6 day-old pupae. If larval DDT-ase is broken down during pupation, the DDT-ase resynthesized in tenerals may be different from larval DDT-ase. The difference observed in response to exposure to radiation between larval DDT-ase and adult DDT-ase may then be caused by a difference in radiosensitivity between the two enzymes. Another explanation for the difference in response is the radiosensitivity of the synthetic pathway and its components. The synthesis of DDT-ase is controlled by the gene *Deh* and differences may exist in the susceptibility of the gene to radiation damage during the various stages of house fly development. The differences are resolved if one compares cell activity in day-old larvae and 5-6 day-old pupae. Cellular metabolic activity will be much higher in rapidly growing day-old larvae than in 5-6 day-old pupae in which cell growth has essentially ceased. Any radiation-induced damage to the genetic material or other vital cell constituents will have a critical effect on further larval development. With the decreased cellular activity in pupae 24 hours before adult eclosion, cellular components would perhaps be less susceptible to radiation damage.

Exposure of house fly pupae to various doses of gamma radiation has been shown to affect adult emergence. Nair (1962) reported gamma-radiation doses of 0.5, 1.0, 2, 2.5, 5, and 10 Krads applied to 30-80 hour-old house fly pupae had no appreciable effect on adult emergence.

In another study, adult house fly emergence from pupae irradiated when 36-48 hours old with 10, 15, 20, and 30 Krads of X-radiation was reduced 6.2%, 22.4%, 53.8%, and 76.8% respectively (Rockstein *et al.*, 1965). Rockstein *et al.* (1967) found no appreciable decrease in emergence of adult house flies X-irradiated as 36-48 hour-old pupae with 2 and 8 Krads. Although no data was collected in this study on the effects of gamma radiation on adult emergence from irradiated pupae, there was no noticeable decrease in adult emergence from 5-6 day-old irradiated pupae.

Also observed in this study was the failure of females to oviposit after exposure as 5-6 day-old pupae to 6, 12, 18, and 24 Krads of gamma radiation which agrees with observations of other authors. Smittle (1967) reported no oviposition by female house flies exposed as 3 day-old pupae to 3 and 6 Krads of gamma radiation. Henneberry (1963) found that female house flies irradiated as pupae with 8 and 16 Krads failed to oviposit after mating with untreated males. Offori (1970) found that female stable flies (*Stomoxys calcitrans*) irradiated as pupae with 3 Krads of gamma radiation 24-18 hours prior to emergence laid no eggs.

Adult house flies exposed as 5-6 day-old pupae to gamma radiation showed increased tolerance to DDT. The difference in LD₅₀ values between flies irradiated with the same dose (Table 9) was possibly due to genetic variation among irradiated individuals, differing degrees of radiation damage, variations in larval and adult rearing conditions, and variations in LD₅₀ test conditions. Temperature, food supply, and overcrowding of the larvae will have some effect on the degree of resistance of the adults to DDT. DDT-treated flies incubated at

30 C will detoxify more DDT than flies incubated at 24 C as DDT displays a negative temperature coefficient of activity (O'Brien, 1967). In this study, house flies irradiated as pupae with 12 or 18 Krads showed increased resistance to DDT. Radiation doses of 6 and 24 Krads increased resistance slightly in one test but had no effect in the second. Varzandeh and Moos (1963) found no difference in response to DDT by X-irradiated house flies. Guenther and Ware (1967) found that irradiation of house fly pupae with 7.5 and 10 Krads resulted in decreased resistance of adults to heptachlor, increased resistance to malathion in females with no change in males, and increased resistance of males to Temik^R (10% formulation of (2-methyl-2-(methylthio) propionaldehyde O-methylcarbamoyl) oxime) with no effect on females. Irradiation of adult house flies with 25, 50, and 75 Krads caused no change in resistance to heptachlor or malathion by either sex whereas resistance to Temik was decreased in both sexes after exposure to 75 Krads. Adult pink bollworm moths (*Pectinophora gossypiella*) irradiated as pupae with 10 Krads of gamma radiation showed decreased resistance to azinphos-methyl (Guthion), a slight increase in resistance to carbaryl, and no difference in resistance to DDT (Rush and Ware, 1969). Moffitt (personal communication) found that newly emerged codling moths (*Carpocapsa pomonella*) irradiated with 25 and 40 Krads of gamma radiation exhibited increased resistance to topically applied azinphosmethyl, carbaryl, and p,p'-DDT. A dose of 40 Krads imparted a smaller increase in resistance to the three insecticides than did the lower irradiation dose (25 Krads). Proverbs (personal communication) found increased resistance to Sevin and Guthion by male codling moths exposed to

sterilizing doses of gamma radiation. Pupae of melon flies (*Dacus cucurbitae*), and Mediterranean fruit flies (*Ceratitidis capitata*) exposed to 10 Krads of gamma radiation 2 days before emergence showed increased resistance to DDT and malathion (Keiser and Schneider, 1969). Male house flies irradiated as pupae 4 days before eclosion with 5 Krads of gamma radiation were shown to be more resistant to malathion than control males. Females were only slightly more resistant than non-irradiated females. Females irradiated with 5 Krads 2 days before emergence were more resistant to malathion than control females whereas males showed little increased resistance (Whitacre and Ware, 1970).

The response of various irradiated species of insects to different kinds of insecticides is quite varied and unpredictable as the above discussion illustrates. Several factors must be considered as important when studying the effects of ionizing radiation on insecticide tolerance in insects: radiation dose and rate of application; insect species and stage irradiated (egg, larval, pupal, adult); experimental conditions; type of insecticide used and method of application. A literature search failed to provide any information on the mode of action of ionizing radiation on insecticide resistance in insects. Ware and co-workers have suggested that increased tolerance to malathion by house flies irradiated as pupae is due to increased levels of carboxyesterases, enzymes responsible for the breakdown of organophosphate insecticides (Whitacre and Ware, 1970; Guenthner and Ware, 1967; Rush and Ware, 1969). Other studies have shown a direct correlation between resistance to malathion and carboxyesterase levels (Matsumura and Brown, 1961). A similar correlation has been shown

between the degree of resistance to DDT and DDT-ase levels in house flies (Lovell and Kearns, 1959). The results of this current study failed to show any increase in DDT-ase levels in gamma-irradiated DDT resistant house flies even though irradiated flies were more resistant than non-irradiated flies. Therefore the mode of action of gamma radiation on house fly resistance to DDT is not the stimulation of synthesis or activity of DDT-ase. Possible alternatives are reduced penetration of DDT through the cuticle or decreased nerve sensitivity to DDT. Irradiation may alter the molecular structure of the cuticle enough to slow down the entry of DDT, allowing the insect more time to detoxify any DDT which enters. One hypothesis proposed for the mode of action of DDT is the formation of a charge-transfer complex with components of the nerve membrane (O'Brien and Matsumura, 1964; Matsumura and O'Brien, 1966a, b). Modification of the molecular framework of the nerve sheath may reduce the formation of charge-transfer complexes between DDT and protein components of the nerve sheath. Future research may demonstrate the above reactions plus other biochemical and physiological processes which contribute to the change in response of house flies to DDT after exposure to sterilizing doses of gamma radiation.

VI. CONCLUSIONS

- (1) DDT-dehydrochlorinase content during DDT resistant house fly development reaches a peak in larvae just prior to pupation and in freshly emerged adults (Table 1). Treatment of teneral adults with the protein synthesis inhibitors puromycin, actinomycin D, or chloramphenicol reduces the synthesis of DDT-ase and α -GPDH indicating that some DDT-ase is resynthesized in young adults (Tables 2 and 3).
- (2) The synthetic pathway of DDT-ase is more susceptible to radiation damage in day-old larvae than in 5-6 day-old pupae. Enzyme activity was reduced in irradiated 3 to 5 day-old larvae irradiated less than 24 hours old (Table 5) but was unaffected in adults irradiated as 5-6 day-old pupae (Table 7). It is suggested that differences in cellular metabolic activity account for the difference in response of DDT-ase to gamma radiation.
- (3) Increased resistance of adults irradiated as 5-6 day-old pupae to DDT (Table 9) is not due to increased levels of DDT-ase but to some other biochemical or physiological factors modified by radiation.
- (4) Gamma radiation doses of 6, 12, 18, or 24 Krads applied to 5-6 day-old pupae completely block oviposition in adults. Day-old larvae exposed to 24 Krads die before pupation. Larvae exposed to 6, 12, or 18 Krads pupate but no adults emerge.

REFERENCES

- Augenstine, L. G. 1962. The effects of ionizing radiation on enzymes. *Adv. Enzymol.* 24: 359-413.
- Brosemer, R. W. 1965a. Changes in glycerophosphate dehydrogenase activity during development of the grasshopper *Schistocerca vaga*. *Biochim. biophys. Acta* 96: 61.
- Brosemer, R. W. 1965b. The effect of puromycin and actinomycin D on the development of grasshopper flight muscle glycerophosphate dehydrogenase. *Biochim. biophys. Acta* 99: 388-390.
- Casarett, A. P. 1968. Radiation biology. Prentice Hall, Inc., New Jersey. 368 pp.
- Daum, R. J. and W. Killcreas. 1966. Two computer programs for probit analysis. *Bull. ent. Soc. Am.* 12(4): 365-369.
- Dinamarca, M. L., I. Saavedra, and E. Valdes. 1969. DDT-dehydrochlorinase-I. Purification and characterization. *Comp. Biochem. Physiol.* 31: 269-282.
- Ebhert, K. H. and D. Heinicke. 1967. Effect of gamma-rays on the action of an enzyme. *Nature, Lond.* 215: 1383-1384.
- Gerhardt, G., and H. Beevers. 1968. Influence of sucrose on protein determination by the Lowry procedure. *Anal. Biochem.* 24: 337-339.
- Gil, L., B. C. Fine, M. L. Dinamarca, I. Balaz, J. R. Busvine, and M. Agosin. 1968. Biochemical studies on insecticide resistance in *Musca domestica* L. *Entomologia exp. appl.* 11(1): 15-29.
- Grigolo, A. and F. J. Oppenoorth. 1966. The importance of DDT-dehydrochlorinase for the effect of the resistance gene kdr in the

- housefly *Musca domestica* L. *Genetica* 37: 159-170.
- Guenthner, A. W. and G. W. Ware. 1967. Effects of X-irradiation on toxicity of malathion, heptachlor, and Temik to the housefly. *J. econ. Ent.* 60(2): 369-373.
- Henneberry, T. J. 1963. Effects of gamma radiation on the fertility and longevity of *Drosophila melanogaster*. *J. econ. Ent.* 56(3): 279-281.
- Henneberry, T. J. 1967. Susceptibility of various stages of *Drosophila melanogaster* to gamma radiation. *J. econ. Ent.* 60(4): 1041-1043.
- Hoyer, R. F. and F. W. Plapp, Jr. 1966. A gross genetic analysis of two DDT-resistant house fly strains. *J. econ. Ent.* 59: 495-501.
- Keiser, I. and E. L. Schneider. 1968. Longevity, resistance to deprivation of food and water, and susceptibility to malathion and DDT of oriental fruit flies, melon flies, and Mediterranean fruit flies sexually sterilized with tepa or radiation. *J. econ. Ent.* 62(3): 663-667.
- Kerr, R. W., D. J. Venables, W. J. Roulston, and H. J. Schnitzerling. 1957. Specific DDT-resistance in house flies. *Nature, Lond.* 180: 1132-1133.
- Khan, M. A. Q. 1969. DDT-dehydrochlorinase and aldrin-epoxidase activity in corn earworm and Polyphemus moth larvae, and house fly adults. *J. econ. Ent.* 62(3): 723-725.
- Kimura, J. and A. W. A. Brown. 1964. DDT-dehydrochlorinase in *Aedes aegypti*. *J. econ. Ent.* 57(5): 710-716.

- Kimura, J., J. R. Duffy, and A. W. A. Brown. 1965. Dehydrochlorination and DDT-resistance in *Culex* mosquitoes. *Bull. Wld Hlth Org.* 32: 557-561.
- Lipke, H. and C. W. Kearns. 1959a. DDT-dehydrochlorinase- I. Isolation, chemical properties, and spectrophotometric assay. *J. biol. Chem.* 234: 2123-2128.
- Lipke, H. and C. W. Kearns. 1959b. DDT-dehydrochlorinase- II. Substrate and co-factor specificity. *J. biol. Chem.* 234: 2129-2132.
- Lipke, H. and C. W. Kearns. 1960. DDT-dehydrochlorinase. *Adv. Pest Control Res.* 3: 253-287.
- Lovell, J. B. and C. W. Kearns. 1959. Inheritance of DDT-dehydrochlorinase in the house fly. *J. econ. Ent.* 52(5): 931-935.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. biol. Chem.* 193: 265-275.
- Marquardt, R. R., and R. W. Brosemer. 1966. Insect extramitochondrial glycerophosphate dehydrogenase: I. Crystalline and physical properties of the enzyme from honeybee (*Apis mellifera*) thoraxes. *Biochim. Biophys. Acta* 128: 454-463.
- Matsumura, F. and A. W. A. Brown. 1961. Biochemistry of malathion resistance in *Culex tarsalis*. *J. econ. Ent.* 54(6): 1176-1185.
- Matsumura, F. and R. D. O'Brien. 1966a. Absorption and binding of DDT by the central nervous system of the American cockroach. *J. agric. Fd Chem.* 14(1): 36-38.
- Matsumura, F. and R. D. O'Brien. 1966b. Interactions of DDT with the components of the American cockroach nerve. *J. agric. Fd*

Chem. 14(1): 39-43.

- Milani, R. 1955. Comportamento ereditario dei caratteri knockdown-resistance (kdr) e plexus (plx) in *Musca domestica* L. Estratto dai rendiconti dell *Inst. Superiore di Sanita* 18: 889-897 (From: Grigolo, A. and F. J. Oppenoorth, 1966).
- Milani, R. 1960. Genetic studies on insecticide resistant insects. *Misc. Publs ent. Soc. Am.* 2(1): 75-83.
- Miyake, S. S., C. W. Kearns, and H. Lipke. 1957. Distribution of DDT-dehydrochlorinase in various tissues of DDT-resistant house flies. *J. econ. Ent.* 50(3): 359-360.
- Moorefield, H. H. 1956. Purification of DDT-dehydrochlorinase from resistant house flies. *Contr. Boyce Thomson Inst. Pl. Res.* 18: 303-310.
- Moorefield, H. H. and C. W. Kearns. 1957. Levels of DDT-dehydrochlorinase during metamorphosis of the resistant housefly. *J. econ. Ent.* 50(1): 11-13.
- Nair, K. K. 1962. Preliminary studies on the effects of gamma-radiation on house fly pupae with special reference to the mechanism of emergence. p. 207-211. *In* Radioisotopes and radiation in Entomology. Int. Atomic Energy Agency, Vienna.
- O'Brien, R. D. and F. Matsumura. 1964. DDT: A new hypothesis of its mode of action. *Science* 146: 657-658.
- O'Brien, R. D. 1967. Insecticides, Action and Metabolism. Academic Press, New York. 332 p.
- Offori, E. D. 1970. Gamma radiation of *Stomoxys calcitrans*. *J. econ. Ent.* 63(2): 574-579.

- Oppenoorth, F. J. 1965a. Some cases of resistance caused by the alteration of enzymes. *Proc. XII Int. Cong. Ent.*, London: 240-242.
- Oppenoorth, F. J. 1965b. DDT-resistance in the housefly dependent on different mechanisms and the action of synergists. *Med. Landbouwhogeschool, Gent* 30(3): 1390-1396.
- Oppenoorth, F. J. and N. W. H. Houx. 1968. DDT resistance in the housefly caused by microsomal degradation. *Entomologia exp. appl.* 11: 81-93.
- Oppenoorth, F. J. and S. Voerman. 1965. A method for the study of house fly DDT-dehydrochlorinase by gas-liquid chromatography. *Entomologia exp. appl.* 8: 293-298.
- Perry, A. S. and W. M. Hoskins. 1950. The detoxification of DDT by resistant houseflies and the inhibition of this process by piperonyl cyclonene. *Science* 111: 600-601.
- Rechsteiner, M. C. 1970. *Drosophila* lactate dehydrogenase and α -glycerophosphate dehydrogenase: distribution and change in activity during development. *J. Insect Physiol.* 16: 1179-1192.
- Rockstein, M., M. Dauer, and P. L. Bhatnagar. 1965. Adult emergence of the house fly *Musca domestica* from X-irradiated pupae. *Ann. ent. Soc. Am.* 58(3): 375-379.
- Rockstein, M., M. Dauer, and P. L. Bhatnagar. 1967. Further studies on the effect of X-irradiation on the house fly *Musca domestica* L. *Radiat. Res.* 31(4): 840-845.
- Rush, R. E. and G. W. Ware. 1969. Toxicity of DDT, carbaryl, and azinphosmethyl to gamma-radiated pink bollworm moths. *J. econ.*

Ent. 62(5): 1124-1125.

- Sawicki, R. M. and A. W. Farnham. 1967. Genetics of resistance to insecticides of the SKA strain of *Musca domestica*. I. Location of the main factors responsible for the maintenance of high DDT-resistance in diazinon-selected SKA strain. *Entomologia exp. appl.* 10: 253-262.
- Sawicki, R. M. and A. W. Farnham. 1969. Examination of the isolated autosomes of the SKA strain of houseflies (*Musca domestica* L.) for resistance to several insecticides with and without treatment with sesamex and TBTP. *Bull. ent. Res.* 59: 409-421.
- Smittle, B. J. 1967. Effect of aeration on gamma irradiation of house fly pupae. *J. econ. Ent.* 60(6): 1594-1600.
- Sternburg, J., C. W. Kearns, and W. N. Bruce. 1950. Absorption and metabolism of DDT by resistant and susceptible house flies. *J. econ. Ent.* 43(2): 214-219.
- Sternburg, J., C. W. Kearns, and H. H. Moorefield. 1954. DDT-dehydrochlorinase, an enzyme found in DDT-resistant flies. *J. agric. Fd Chem.* 2(22): 1125-1130.
- Sternburg, J., E. B. Vinson, and C. W. Kearns. 1953. Enzymatic dehydrochlorination of DDT by resistant flies. *J. econ. Ent.* 46(3): 513-515.
- Tombes, A. S. and A. J. Forgash. 1961. DDT-dehydrochlorinase in the Mexican bean beetle, *Epilachna varivestis* Muls. *J. Insect Physiol.* 7: 216-223.
- Tsukamoto, M., T. Narahasi, and T. Yamasaki. 1965. Genetic control of low nerve sensitivity to DDT in insecticide-resistant

- house flies. *Botyu-Kagaku* 30: 128-132.
- Ursprung, H., K. D. Smith, W. H. Sofer, and D. T. Sullivan. 1968.
Assay systems for the study of gene function. *Science* 160:
1075-1081.
- Varzandeh, M. and W. S. Moos. 1963. The effect of X-irradiation on
longevity, emergence, and DDT-susceptibility of the house
fly. *Proc. N. Cent. Br. ent. Soc. Am.* 18: 55-59.
- Wagoner, D. E. 1967. Linkage group karyotype correlation in the
house fly determined by cytological analysis of X-ray induced
translocations. *Genetics* 57: 729-739.
- Whitacre, G. K. and G. W. Ware. 1970. Effects of gamma- and X-
irradiation on the toxicity of malathion to house flies.
J. econ. Ent. 63(2): 424-426.
- Wiesmann, R. 1947. Untersuchungen uber das physiologische Verhalten
von *Musca domestica* L. verschiedener Provenienzen. *Mitt.*
schweiz. ent. Ges. 20: 484-504. (From: Hooper, G.H.S. 1967.
A new metabolite of DDT in *Culex pipiens fatigans* Wied.
Proc. R. Soc. Queensland 79(2): 9-16.)
- Winstead, J. A. and T. G. Reece. 1970. Effects of gamma radiation
on the chemical and physical properties of lactate dehydrogenase.
Radiat. Res. 41: 125-134.
- Yanda, V. E. 1968. Simple correlation and plotting package CS022.
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